

CLAIMS

1. Method for increasing the efficiency of targeted integration of a polynucleotide to a pre-determined site into the genome of a filamentous fungal cell with a preference for NHR, wherein said polynucleotide has a region of homology with said pre-determined site, comprising steering an integration pathway towards HR.
2. The method of claim 1, wherein the steering comprises providing a mutant of a parent filamentous fungal cell, wherein the ratio of NHR/HR is decreased in the mutant as compared to said ratio in said parent organism measured under the same conditions.
3. The method of claim 1 or 2, wherein the steering comprises providing a mutant which is deficient in a gene encoding a component involved in NHR, and/or has a decreased level of a component involved in NHR.
4. The method of claim 3, wherein the mutant is, preferably inducibly, deficient in at least one of the following genes: *hdfA* or homologues thereof, *hdfB* or homologues thereof, or both, and/or has, preferably inducibly, a decreased amount of at least one of the proteins encoded by these genes.
5. The method of claim 3 or 4, wherein a gene involved in NHR has been replaced by a non-functional variant.
6. The method according to any one of claims 1 to 5, wherein the steering comprises adding an excess of small double stranded polynucleotides to the polynucleotide to be integrated.
7. The method according to any one of claims 1 to 6, wherein the steering comprises decreasing the activity of at least one protein active in the NHR by adding an inhibitor of said protein(s).

8. The method according to any one of claims 1 to 7, wherein the mutant has an increased level of a component involved in HR.
- 5 9. The method according to any one of claims 1 to 8, wherein a filamentous fungal which has a ratio NHR/HR less than 50, preferably less than 10, even more preferably less than 1, and most preferably less than 0.001 is used.
- 10 10. A mutant of a parent filamentous fungal cell, the parent organism having a preference for NHR, wherein the ratio of NHR/HR is decreased in the mutant as compared to said ratio in said parent organism measured under the same conditions.
- 15 11. The mutant according to claim 10, wherein the mutant is deficient in a gene encoding a component involved in NHR, and/or has a decreased level of a component involved in NHR.
- 20 12. The mutant according to claim 10 or 11, wherein the mutant is, preferably inducibly, deficient in at least one of the following genes: *hdfA* or homologues thereof, *hdfB* or homologues thereof, or both, and/or has, preferably inducibly, a decreased amount of at least one of the proteins encoded by these genes.
- 25 13. The mutant according to any one of claims 10 to 12, wherein in the genome of the organism a gene involved in NHR has been replaced by a non-functional variant.
- 30 14. The mutant according to any one of claims 10 to 13, wherein the mutant has an increased level of a component involved in HR.
15. The mutant according to any one of claims 10 to 14, wherein the mutant is a recombinant mutant.
16. A filamentous fungal which has a ratio NHR/HR less than 50, preferably less than 10, even more preferably less than 1, and most preferably less than 0.001.

17. The filamentous fungus according to any one of claims 10 to 16 transformed with a DNA construct comprising a DNA sequence comprising a gene of interest encoding a polypeptide of interest.
- 5
18. The filamentous fungus according to any one of claims 10 to 17, wherein the filamentous fungus is an *Aspergillus*, *Penicillium* or *Trichoderma* species.
19. The filamentous fungus according to claim 18, wherein the *Aspergillus* is an
- 10 *Aspergillus niger* or an *Aspergillus oryzae* species.
20. The filamentous fungus according to claim 18, wherein the *Penicillium* is a *Penicillium chrysogenum* or *Penicillium citrinum* species.
- 15
21. Method for producing a polypeptide of interest, wherein the filamentous fungus of any one of claim 10 to 20 is used.
22. Method for producing a metabolite, wherein the filamentous fungus of any one of claim 10 to 21 is used.
- 20
23. Method according to claim 22, wherein the metabolite is a carotenoid compound or a beta-lactam compound.
24. Isolated DNA sequences having SEQ ID NO: 2 or 5 or 19 or 22 or homologues thereof.
- 25
25. Isolated polypeptides encoded by the DNA sequences of claim 24 or homologues thereof.